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Arborization Patterns of Cervicovaginal Mucus of Sheep

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Arborization Patterns of Cervicovaginal Mucus of Sheep[1]

DONALD L. DANIEL[2], DONALD S. BELL[3] AND VANCE L. SANGER[4]

INTRODUCTION

In the past, investigators have noted variations in cervical mucus secretions in the human female, the cow, and the ewe. However, it was not until 1946 that a description of the arborization patterns of human cervical mucus was presented by Papanicolaou (9). Since this original description was published, Pommerenke and Viergiver (11) related the association between arborization patterns and the menstrual cycle.

Sheep research dealing with arborization patterns in cervical mucus has been very limited. The only work known to be available originated at Massey Agricultural College. In reviewing this work, a striking similarity could be detected in the results of the cervical mucus arborization pattern studies in the human, the bovine, and the ovine species.

Due to the small size of the ewe, the size of the reproductive tract and its position, and the form and size of the cervix, the techniques employed in obtaining mucus samples in the human female and the cow are not readily applicable to the ewe.

The objectives of this study were to determine: (1) if arborization patterns were manifested by cervicovaginal mucus of ewes taken from the region of the os uteri, and (2) if there was any pattern variation in the various phases of the estrous cycle and in anestrus.

EXPERIMENTAL PROCEDURE

Management of Experimental Animals

Eight multiparous Columbia ewes (4 and 5 years old) were involved in the study. From July 22 to October 9, the ewes had access to an open-end shelter adjacent to the pastures. The pastures were of two types: bluegrass (*Poa pratensis*) and ladino clover (*Trifolium repens var. ladino*). From October 9 to December 1, the ewes were confined

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[1]Based on a thesis submitted by the senior author in partial fulfillment of the requirements for the Master of Science degree, The Ohio State University. A report of this work was presented at the American Society of Animal Production Meeting, November 25-26, 1960.

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to the barn and received a daily allowance of mixed hay, grain, and legume silage.

Throughout the study, vasectomized rams fitted with aprons to prevent coitus were used to detect estrus in the ewes. The rams were stabled separately from the ewes but were turned in twice daily at 8:00 a.m. and 4:00 p.m. Each teasing period lasted 30 to 45 minutes.

Procedure for Taking Mucus Samples

To obtain a mucus sample, the ewe was placed on a stanchion platform fitted with sides. The external genitalia were washed with a commercial detergent and cold water and dried. A glass speculum (30.48 cm. in length x 2.54 cm. in diameter) was lubricated with a neutral lubricant.[5] The speculum, after being inserted into the vagina, was passed the full length of the vagina and then withdrawn slowly until the cervix appeared. An ordinary flashlight supplied the necessary light for observation.

A disposable plastic insemination tube (1 mm. inside diameter x 5 mm. outside diameter x 40.64 cm. in length) was placed via the speculum at the os uteri. The tube was then rotated in the area of the external orifice of the cervix to obtain a sample of the mucus. In the withdrawal of the insemination tube, care was taken to prevent contamination with any mucus which might be present on the floor of the speculum. A clean tube was used for each sample.

Slide Preparation

Immediately upon obtaining the mucus, it was spread evenly and thinly on a clean slide, with the insemination tube used as an applicator. In applying the mucus to the slide, the applicator was pressed firmly against the slide to eliminate irregularities in mucus thickness. The slides were then air-dried for approximately 5 hours.

After the drying period, the slides were examined through a stereomicroscope with reflected light. To preserve the various patterns for more than 12 hours, they were stored in a glass vacuum-type desiccator containing calcium chloride.

Sampling Periods for Cervicovaginal Mucus

Throughout the first half of this study, cervicovaginal mucus smears were taken three times a week from eight ewes: four grazing on each pasture. This phase encompassed a part of the anestrous period, July 10 to August 17, and the early estrous cycles, August 19 to October 9.

Four of the original eight ewes were used to gain a more detailed description of the variation of mucus patterns associated with the estrous

[5]K-Y Sterile Lubricant, Johnson and Johnson, New Brunswick, New Jersey.

period. These four ewes, with a previously established heat cycle date, were subjected to teasing. Immediately after teasing, cervicovaginal mucus smears were made every 4 hours for a period of approximately 52 hours prior to estrus, throughout estrus, and 32 hours post-estrus. To avoid sampling error, two separate samples were taken and a slide was made from each sample.

RESULTS

Arborization Pattern Types

During the study, four distinct pattern types were exhibited by the cervicovaginal mucus. These pattern types were very similar to those first described by Papanicoloau (9). The four characteristic pattern types were: amorphous, rosette, arbor, and fern (Figures 1, 3, 5, and 9).[6]

In addition to these characteristic pattern types, intermediate types were exhibited. The variation in the amorphous pattern was due to concentration and size of the crystals (Figures 1 and 2). The rosette, arbor, and fern patterns varied as to the number of branches, sub-branches, configuration, and overall size (Figures 3 through 10). It was found that more than one pattern type could occur on the same slide (Figures 3, 5, and 7).

Anestrus and Estrus

In the period immediately preceding the normal autumn estrous cycles, the rosette and arbor patterns tended to appear in a 17-day cyclic manner, interspaced by the amorphous pattern type. These periods of arborization extended over a time interval of 3 to 7 days and the 17-day period coincided with the "expected estrous date." The "expected estrous date" was calculated by subtracting 17 days, or multiples thereof, from the date of the first established estrus.

The proestrous period was generally characterized by the rosette and/or arbor patterns. The fern pattern appeared at the beginning of estrus and was replaced at mid-estrus by the rosette and/or arbor pattern. Near the end of estrus, the fern pattern occasionally re-appeared, followed again by the rosette and/or arbor pattern. The amorphous pattern type appeared very abruptly at the cessation of estrus.

Metestrum and diestrum were characterized by the amorphous pattern type. Arborization (rosette, arbor, and fern patterns) occupied 0.5 and 2.5 days of the entire estrous cycle and the amorphous pat-

[6]A Bausch and Lomb model L photomicrographic camera equipped with a 32 mm. microtessar lens, combined with oblique substage lighting furnished by a 200-watt spotlight, was used for all photomicrographs.

tern type occupied the remaining 16.5 to 14.5 days of the usual 17-day cycle.

Viscosity of Vaginal Secretions

At estrus there was a copious flow of clear, watery, and stringy fluid from the speculum after its insertion into the vagina. Immediately post-estrus, however, the viscosity of the fluid increased while the volume decreased. This trend continued in a progressive manner through both metestrum and diestrum until the smears appeared thick or viscous and white in color. A reversal of this occurred in proestrum, with rapid progress towards the clear watery phase at the onset of estrus. These observations are consistent with those of Sanger *et al* (13) and Schindler *et al* (14).

Sterile Lubricant and Saline Solution

To avoid any possibility of the sterile lubricant causing or modifying the arborization patterns, the lubricant was tested alone and with various concentrations (0.1 percent to 0.6 percent) of normal saline solution. The lubricant alone did not give arborization patterns. However, atypical arbor and fern patterns were found at the higher saline concentrations (Figures 11 to 14). In comparison, drying time and arborization of cervicovaginal mucus required 5 hours at room temperature and the lubricant and saline mixture required 9 hours at room temperature.

DISCUSSION

Pattern Type Variation

In the description of the pattern types, variations within a given pattern type were noted. Pattern type variation was also described by Campos da Paz (4). He distinguished two distinct fern types and referred to them by names of two Brazilian ferns which they resembled: Samambaia, which has a rigid geometrical form, and Selaginela, which has a less symmetrical form. Samambaia and Selaginela correspond respectively to the regular and fine, full fern described in this study.

Intra-pattern type variations were usually individually distinct. By association, one type could easily be distinguished from the other. However, inter-pattern relations arose because of two factors: one of size and the other of fullness (number of branches and sub-branches). For example, differentiation between a large rosette and a small arbor in a number of cases became difficult. The transient form of a fine, full arbor in many aspects appeared to be a near-duplicate of a fine, full fern. The solution to this dilemma was frequent observation of a large number of patterns.

Variation in pattern types could be attributed to a number of factors acting either individually or in combination. For example, the regular fern was found only in the heavier mucus portion of the slides, usually at the ends of the slides. The fine, full fern was characteristic of the thinly spread cervicovaginal mucus. These observations are consistent with those of Zondek and Cooper (17).

During this study, considerable difficulty was encountered in trying to maintain various arborization patterns over a period of days. The most difficult periods were days of high humidity when a pattern such as a fine, full arbor would completely disintegrate into the amorphous pattern type. The same diffusion or disintegration could be accomplished in some cases by exposing the slide to the moisture from the super-imposed palm of the hand. Under the same circumstances, intra-pattern type transition was observed to occur. For example, a semi-fine, full rosette as shown in Figure 5 could diffuse and form a more extreme type of fine, full rosette as in Figure 4.

Besides disintegration and transition of the various patterns, there was a problem of inhibition. It was found that rapid desiccation of newly made smears in a desiccator containing calcium chloride produced an atypical pattern type unsuitable for study. Abou-Shabanah and Plotz (1) described similar phenomena such as disintegration and inhibition which occurred in their study of the biochemical basis of arborization.

Anestrus and Estrus

During the anestrous period of late July and August, the rosette and arbor patterns occurred with a great deal of regularity at intervals of approximately 17 days. The ewes did not accept the ram at any time during the anestrous period. This evidence of cycling in the supposedly quiescent period may indicate a "sub-level cycling" prior to the regular estrous cycle.

Estrus in the Columbia ewe is characterized by the rosette, arbor, and fern patterns. However, the fern pattern seems to be associated only with the estrous period, occurring at the beginning of estrus and occasionally towards the end of estrus. Since samples were taken every 4 hours, the possibility of missing the occurrence of the latter fern was increased because it seemed to occur for only a short period of time. Usually arborization patterns were replaced by the amorphous pattern type within 4 hours after the ewe failed to show signs of estrus.

In previous work dealing with human, bovine, ovine, and porcine cervical mucus smears, the quantity of a particular pattern type was the criterion for the determination of the phase of the menstrual or estrual cycle.

The results of this study indicate a qualitative rather than a quantitative approach to the pattern types of cervicovaginal mucus of sheep in order to determine the phase of the estrous cycle. A justification of the qualitative approach might be found in the time interval in which the fern pattern appears in cervical mucus. In humans, the fern pattern appears from the 5th or 7th day to the 20th or 22nd day of the menstrual cycle, as reported by Roland (12). Alliston *et al* (2) reported that the first indication of the fern-like pattern in cattle became evident about 3.5 days before estrus. McDonald and Raeside (8) found arborization in the cervical mucus of normal cycling ewes to be present mainly near the time of estrus. However, arborization during the estrous cycle of all ewes ranged from 6.5 days prior to estrus until 4.5 days post-estrus. Betteridge and Raeside (3) observed that in seven of ten sows, arborization appeared from 1 to 7 days during an 8-day period commencing 3 days before estrus.

The Columbia ewes in this study exhibited the fern pattern at the onset and usually at the end of estrus. The total estrous period lasted approximately 40 hours. Thus, the arborization pattern phenomenon in these sheep occurred almost simultaneously with the estrous period and changed markedly with the various phases of the estrous period.

SUMMARY

Four distinct pattern types, classified as amorphous, rosette, arbor, and fern, were observed in dried cervicovaginal mucus of Columbia ewes. Anestrus was characterized by the arbor and the rosette patterns, which tended to appear in a 17-day cyclic manner, interspaced by the amorphous pattern type. Estrus was characterized by the rosette, arbor, and fern patterns. The fern pattern seemed to be associated only with the estrous period. Metestrum and diestrum were normally characterized by the amorphous pattern type.

Evidence was obtained which shows that some techniques used by the operator could bring about transition, disintegration, and inhibition of the arborization patterns.

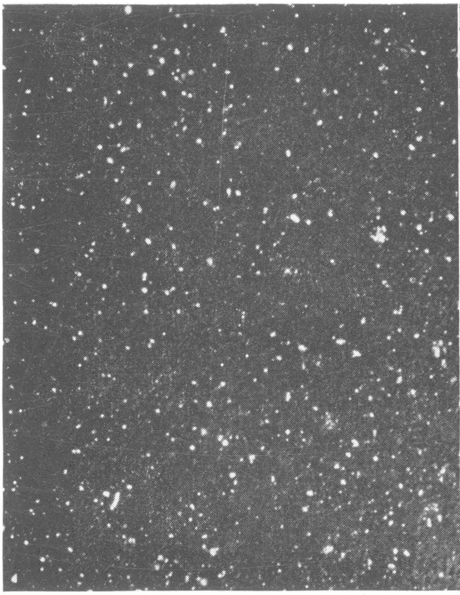

Fig. 1.—Amorphous; 23x.

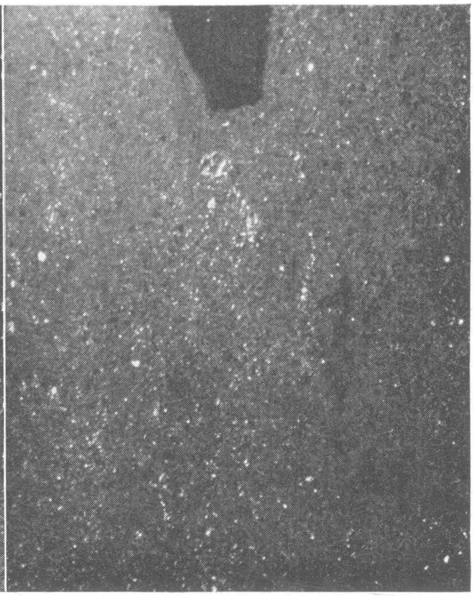

Fig. 2.—Fine amorphous; 23x.

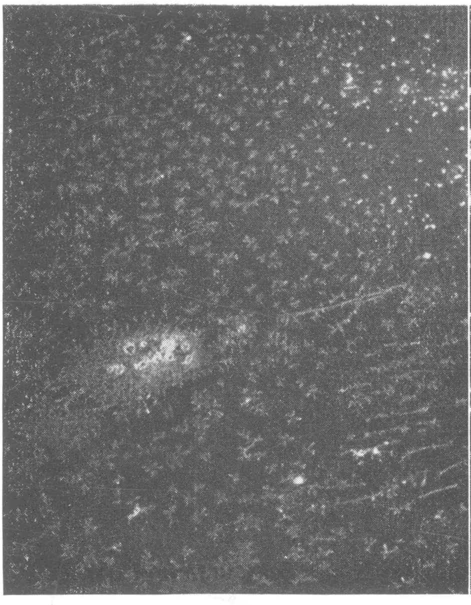

Fig. 3.—Rosette; 23x.

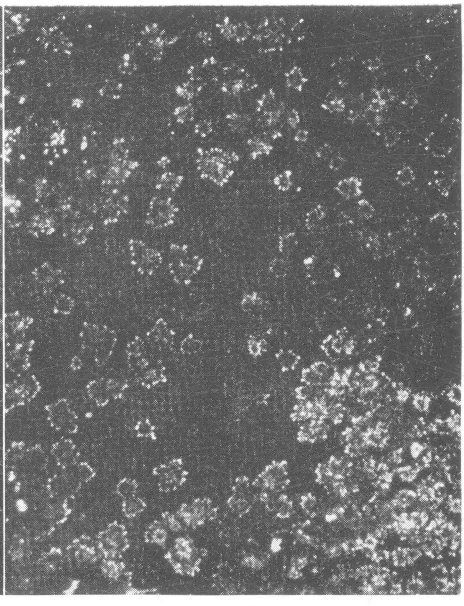

Fig. 4.—Fine, full rosette; 23x.

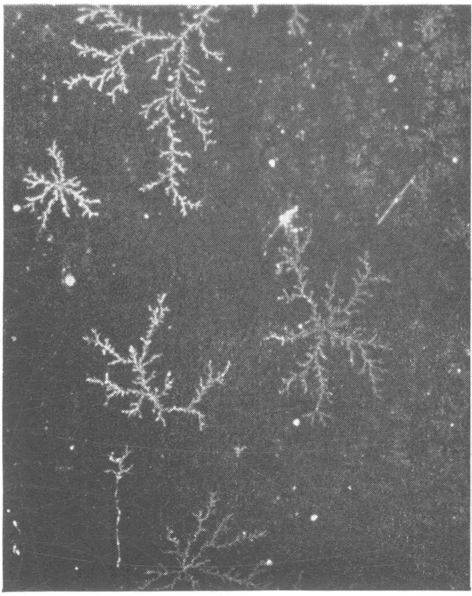

Fig.5.—Arbor with semi-fine, full rosette; 23x.

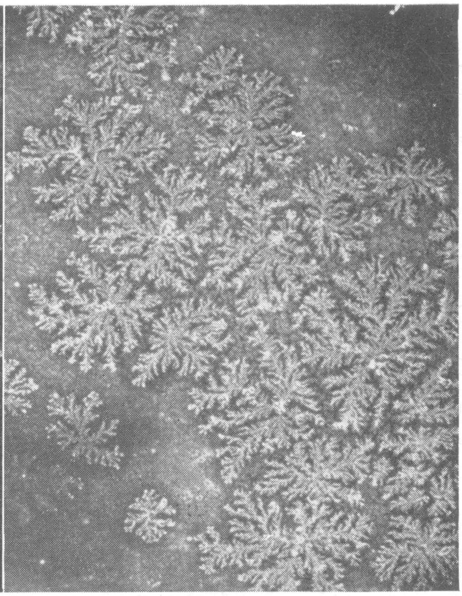

Fig. 6.—Fine, full arbor; 23x.

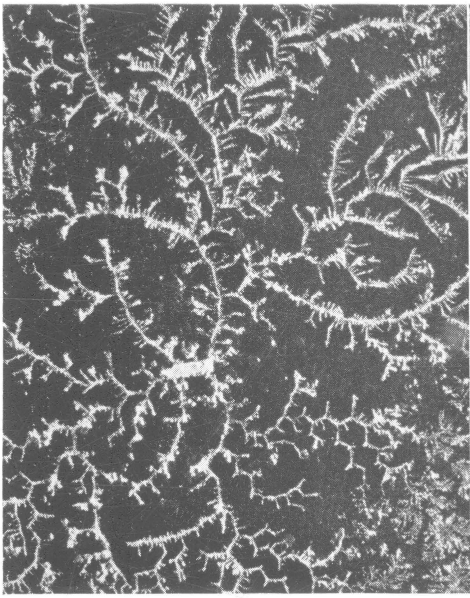

Fig. 7.—Rambling arbor; 43x.

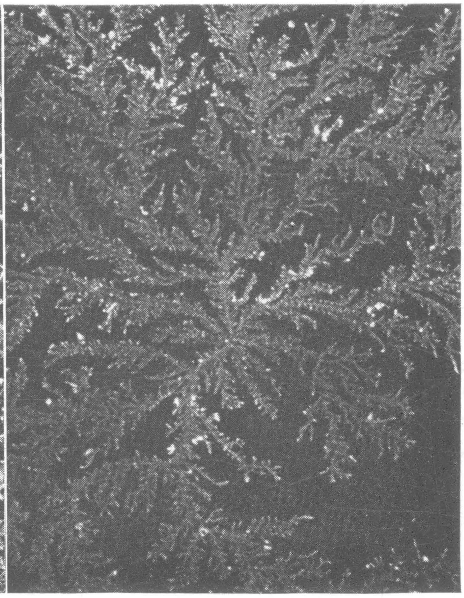

Fig. 8.—Rambling, fine, full arbor, 23x.

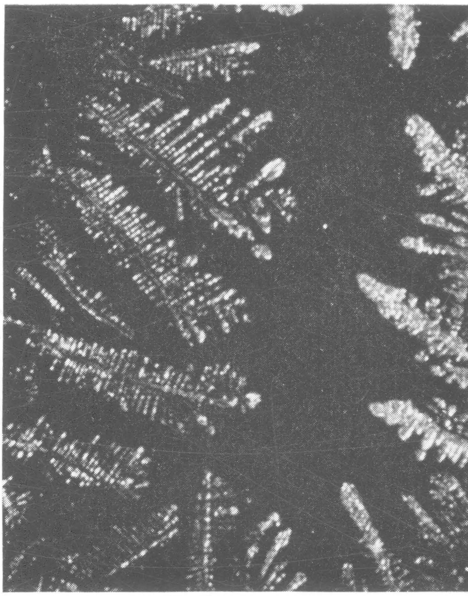

Fig. 9.—Fern; 37x.

Fig. 10.—Fine, full fern; 37x.

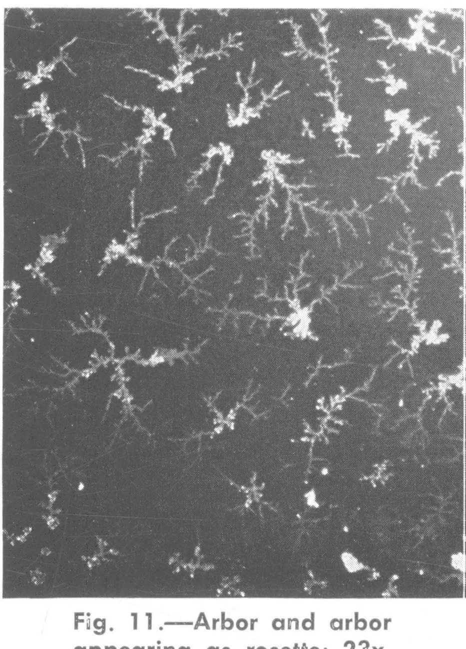

Fig. 11.—Arbor and arbor appearing as rosette; 23x.

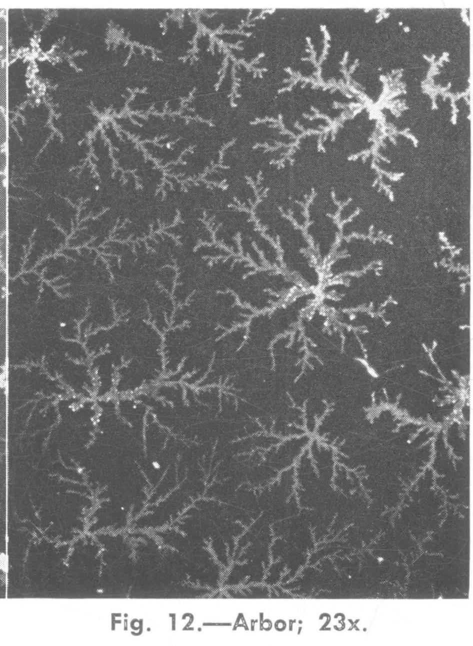

Fig. 12.—Arbor; 23x.

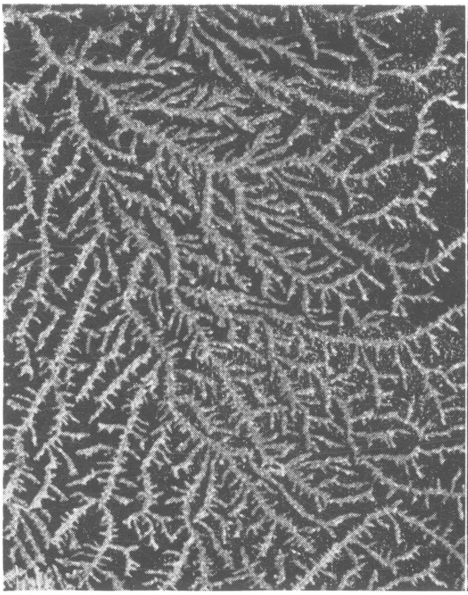
Fig. 13.—Rambling arbor; 23x.

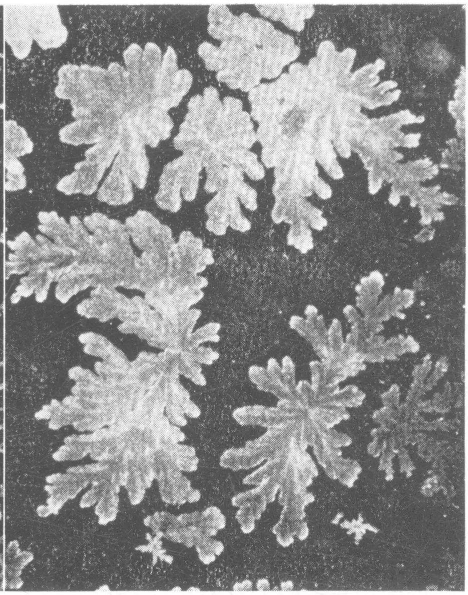
Fig. 14.—Fern; 23x.

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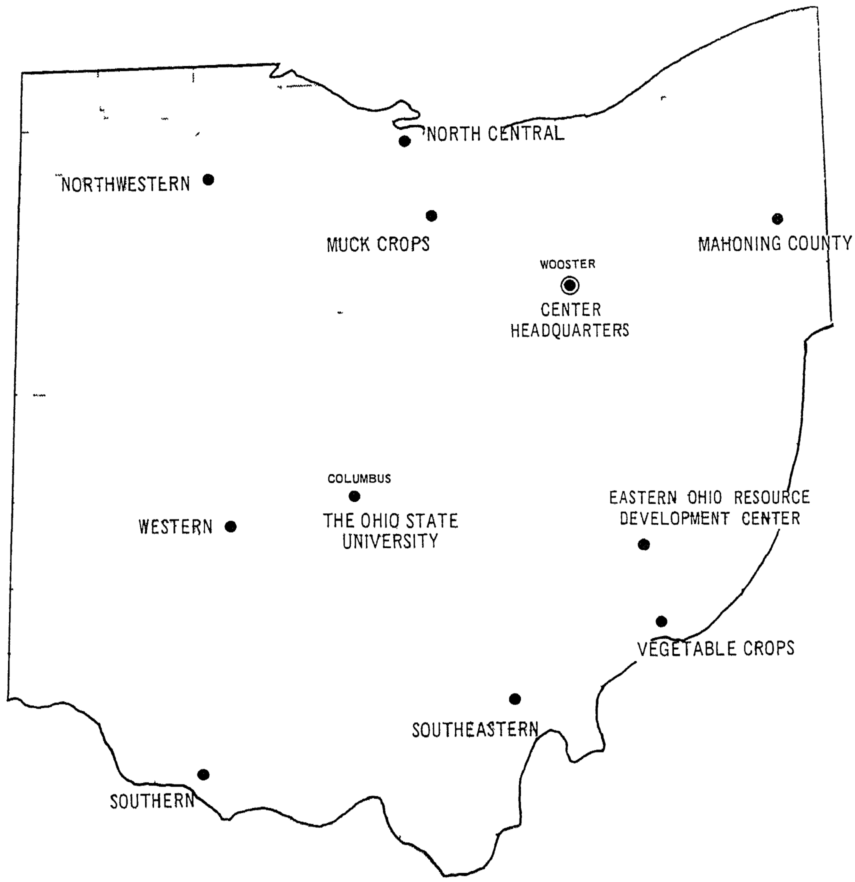

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Northwestern Branch, Hoytville, Wood County: 247 acres
Southeastern Branch, Carpenter, Meigs County: 330 acres
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Western Branch, South Charleston, Clark County: 428 acres